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The Effects of Temperature and Leaf Sugar Content on the Electrical Conductance of Strawberry Leaf Exudates

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THE EFFECTS OF TEMPERATURE AND LEAF SUGAR CONTENT ON THE ELECTRICAL CONDUCTANCE OF STRAWBERRY LEAF EXUDATES

By

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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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KCS.

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INTRODUCTION

Man has observed for innumerable years the effect of low temperatures on growing vegetation with the resulting injury and death. Once considered to be the inevitable fate of plant life exposed to low temperatures, this injury is now subject to partial control or alteration under certain conditions (12). These conditions involve a genetic change in the plant protoplasm by breeding a hardier, more resistant variety or by a change in the environment of the plant that induces resistance to low temperatures. The latter change is frequently known as "hardening" of the plant, a term used to denote this process, whether naturally or artificially induced. While the study of these changes (32) has given considerable insight into the subject, there is little understanding of the basic cause or causes of frost injury, nor is the subsequent resolution of the problem known.

The bulk of recent scientific investigations into winter injury has been mainly concerned with plant physiology (21). The detection of measurable differences in the physiological factors under various conditions has offered a means of explaining frost resistance, at least partially. This thesis is a report of a continuation of an investigation into this aspect of the problem. Strawberries were selected for this research because of their susceptibility to frost injury, comparative ease of control for research purposes, and practical significance as a crop in this area. Previous studies at this college (20) have revealed the desirability of applying a protective mulch when the plants have reached a maximum level of stored carbohydrate reserve as determined by sugar analysis. Investigations elsewhere with various plant materials have indicated that numerous other factors including mineral content and permeability of the cell, are involved (9,27,32). The objective of this study then is to examine some of these factors with particular reference to the strawberry plant.

LITERATURE REVIEW

Some of the first scientific investigations into frost injury were made by Goppert (11). During mid-winter he brought indoors plants which had survived the low winter temperatures and, after exposing them to higher indoor temperatures for several days, observed that they were killed by a return to the colder environment. Since then the effect of naturally and artificially produced temperatures on various plants has been studied extensively (14,21). The accumulated research dealing with plant injury and resistance to low temperatures renders a single explanation of these phenomena untenable. Low temperature alone does not necessarily cause death, as Lipman (24) kept seeds and bacterial spores at temperatures only slightly above absolute zero for forty four hours without adverse effects.

Rosa (31) and Levitt (21) have reviewed this phase of plant physiology thoroughly including the earlier theories of frost injury. Of these theories only the rupture theory persists. Bugaevsky (3) states that the formation of cavities in the roots of the carrot and other vegetables results from rupture by pressure developed as the tissue water freezes. This rupturing damages the cell membrane.

On the basis of modern evidence several explanations regarding the causes of freezing injury in plants have been proposed. Molisch (28) advanced the idea that ice formation results in water removal from the cell with consequent dehydration causing cell death. Wiegand (39) studied the condition of buds and twigs in the winter and modified Molisch's idea to include a critical evaporation point for each cell. Stiles, (35) on the basis of observations of both living and nonliving colloidal systems undergoing freezing, held that frost death results when large ice crystals in the protoplasm cause an aggregation of particles normally in a dispersed phase. This condition is usually irreversible. Maximow (26) maintained that the pressure due to ice formations distorts the cell with rupture of the protoplasm. This pressure concept was substantiated by Chandler and Hildreth (5) and by Dexter (6). The latter noted that hardy leaves were less severely damaged in a hydraulic press than the tender leaves of the wheat plant. Iljin (18) contended that the death of tissues occurs in thawing. Preceding cooling, he explains, there is a loss of water with reduction in the size of the vacuoles with resulting plasmolysis. Harvey (12) studied the precipitation of proteins in the juice of hardened and unhardened cabbage plants. Increased precipitation in the unhardened plants is explained as an injurious effect of increased electrolytes on the proteins. During the hardening period, complex proteins are often changed to less easily coagulated forms as evidenced by an increase in amino nitrogen. Scarth and Levitt (32) believe that permeability is the factor directly related to injury and indirectly to resistance. Increased permeability of the cell membrane permits an exodus of water from inside the cell. This is advantageous to the cell, since intra-cellular freezing with disruption of the protoplasm is almost always fatal.

In general these various theories agree that ice formation initiates changes in the cell constituents. Whether this formation occurs intracellularly or extracellularly depends on external factors as well as the inherent properties of the particular plant cells. The chemical or mechanical effects of these ice formations are not sufficiently known to warrant a single explanation.

Hardiness is the mechanism through which the plant either resists or overcomes the effect of frost injury. The environmental factors which can cause hardiness are numerous, but in general they are characterized by their ability to check plant growth. The increased resistance in cabbage plants conferred by low temperature was studied extensively by Harvey (12). Exposure to 3° centigrade for five days prevented freezing at -3° centigrade for one-half hour. From later observations (14) he concluded that hardiness occurs below a critical or threshold temperature of about 5° centigrade and is essentially a cold shock response. However, Peltier and Tysdal (29) found that a long exposure time is important in improving resistance in alfalfa. Angelo and others (1) found continuous low temperatures conducive to hardening strawberry plants. Thus, although temperature alters hardiness, there exist many differences among plants in their response to exposure time and temperature.

The effect of moisture on hardening has been investigated by Chandler (4) and Rosa (31), who found that droughty conditions increased frost resistance. Angelo and others (1) noted under experimental conditions similar results with strawberries supplied with varying amounts of water.

The sugar content of plants increases upon exposure to low temperatures. This long recognized fact has stimulated considerable investigation because, as already mentioned, exposure to low temperature may also increase frost resistance. Hildreth (17) observed a sharp increase in total sugars of two apple varieties from October to December. Rosa (31) and Lazuruk (20) found an increase in sugar content of several varieties of strawberries as fall progressed. This increase in sugar content is often attributed to a starch to sugar conversion. Harvey (12) and Rosa (31) each observed an increase in monosaccharides and disaccharides and a decrease in polysaccharides upon hardening at low temperatures.

Work which has been conducted to study the relationship of carbohydrate content to varietal hardiness is conflicting. Hildreth (17) and Stuart (36) found differences in sugar content between hardy and nonhardy apple varieties. Harvey (13) found higher sugar contents among the hardier red varieties than in the more tender green types of cabbage. The application of sugar solutions to plants has produced little effect on cold resistance (4,7). Sugar content apparently is a factor in hardiness, but not to a degree where it has been demonstrated as a basic factor.

Nitrogeneous substances have long been investigated in their relation to the cold endurance of plants. Harvey (12) detected an increase in total and amino nitrogen during the hardening process in cabbage. Dexter (7) found that hardening plants with limited quantities of light increased their soluble nitrogen content. Lott (25) found that hardiness in brambles correlated with protein content. Siminovitch and Briggs (33) have established a positive correlation between water soluble protein and degree of hardiness in the bark of the black locust tree. Recently Levitt (22) has suggested that a sugar-protein complex (a muco-protein) is involved in frost hardiness. He found the carbohydrate contents of the protein fractions of hardened cabbage significantly different from those of the unhardened. He says that an initially high sugar content in the protein fractions may be required for the plant to develop frost resistance.

The water content of the plant appears to be an important factor in frost resistance. Hildreth (17) observed a decrease in moisture content of apples as winter approached, noting that the hardier variety remained more nearly constant during the winter. Harvey (12) and Rosa (31) each observed decreases in moisture when cabbage plants were undergoing hardening. Since decreased moisture content would also affect cell sap concentration, the latter has been studied extensively. Chandler (4) found that sap densities as determined by freezing point bore a positive correlation to hardiness in numerous garden plants. The viscosity of the pressed juice of small grains has shown a correlation with hardiness (37). Levitt and Scarth (23) using a plasmolytic method of determination, found a greater osmotic pressure in a hardy apple variety than in a semi-hardy variety.

Since field trials often take considerable time and weather conditions are variable, Dexter, Tottingham and Graber (8) sought a rapid means of measuring the relative degree of cold resistance. They maintained that because frost injury involves an increase in cell permeability the quantity of electrolytes which diffuse out of plant tissue into water can be measured by conductivity tests and is proportional to the injury. In further work (9) they investigated alfalfa roots dug from frozen ground and placed in water at 25° centigrade for ten hours. Expressing specific conductivity in reciprocal ohms they found a lower conductivity among hardier varieties. Experiments on grains grown in greenhouses and later frozen in an air chamber yielded similar results, but evidence was considered insufficient to determine the correlation of hardening with electrical conductivity. Megee (27) found a substantial difference in conductivity between hardy and nonhardy alfalfas in the late fall.

Ivanov (19) measured the electrical conductivity of the pressed juice of wheat and cabbage and noted an increase in electrolytes before any perceptible frost injury. The amount increased with decreasing temperature until death of the plant and then remained constant. The less hardy wheat varieties gave the greater increase. Conversely, Levitt and Scarth (23) using the plasmolytic method observed an increase in permeability of cabbage seedlings to polar substances, including water, upon exposure to hardening temperatures.

Using the electrolytic method, Emmert and Howlett (10) determined the resistance of fifty five apple varieties to low temperatures. They found the Garnet crabapple hardier than the McIntosh apple in the fall, but they found the reverse to be true in the late winter and early spring. Thus the time of testing appears to be an important factor. Wilner (40) in his studies with woody plants has found that values of electrical conductance were not modified by seasonal variations or cultural practices.

STUDIES WITH FIELD PLANTS

This investigation is divided into two main sections. The first section is concerned with the study of the electrical conductance of the leaf exudates as affected by sugar content and the seasonal development of hardiness in field plantings. The second section is a further study using greenhouse plants subjected to several controlled conditions.

Preliminary Studies of the Electrical Conductance Method

Experimental

Determinations of electrical conductance were begun in the fall of 1954 using several varieties of strawberries. On October 25 leaves were collected from field plants of the Erie variety. These and the subsequent collections were made in the mid-morning to minimize differences resulting from changes in photosynthetic activity according to the time of day. Leaves selected were those which were exposed to the sun rather than those shaded by other leaves, uniform in size and color, and free of any visible injury or defects. Immediately they were taken to the laboratory and, after removing the petioles, 15 gram samples of whole leaves were weighed in duplicate using an analytical balance. They were then carefully washed free of soil particles and surface debris by immersing several times in tap water and were finally rinsed with distilled water. The samples were air dried at room temperature until the surface water had evaporated, placed in moistureproof bags, and stored in freezing chamber at 10° Fahrenheit for 24 hours.

Following this treatment the samples were transferred to 400 milliliter beakers containing 250 milliliters of distilled water. The leaves were agitated until their surfaces were under the water after which the beakers were covered with watchglasses. At the end of seven hours at room temperature a portion of the water extract was removed from each beaker and its resistance measured using a Wheatstone bridge and a standard conductivity cell. Specific conductance for each sample extract was then calculated. Subsequent collections and determinations were made on November 6, 10, and 18, at which times other varieties were included. All eleven varieties in the plot were sampled on November 23 at which time additional amounts of leaves were collected for sugar analysis. These additional leaves were dried in an air-blast oven at 75° centigrade for 48 hours, ground in a Wiley mill, and stored in capped bottles until analyzed. Reducing and total sugars were determined by the Hassid modification of the Hagedorn-Jensen method (15,16).

Results

Table 1 represents the specific conductance values of the leaf exudates for the several varieties tested in 1954. It will be noted that there is a fairly consistent agreement between duplicates of each variety. Unfortunately Erie was the only variety examined over the entire period. The values for Erie show a marked decrease in conductivity as the fall season progressed with a slight increase occurring on the last sampling date. The other varieties seem to indicate a similar trend insofar as they were tested.

In Table 2 the data is presented of the specific conductance and sugar content of the November 23 leaves. There is an unusually great variation in sugar content among the varieties. Another sampling, if

Table 1. The Electrical Conductance of Leaf Exudates from Several Varieties of Strawberries During the Fall of 1954.

Date of Sampling	Variety: Erie	Premier	Sen. Dunlap	Red Rich
	Specific Conductance*			
Oct. 25	1.13	-	-	-
Nov. 6	.87 .78	.60 .55	.50 .53	-
Nov. 10	.48 .43	.54 .61	.39 .40	.60 .67
Nov. 18	.44 .41	-	-	-
Nov. 23	.41 .48	.64 .64	.42 .49	.64 .61

* Specific Conductance in ohm^{-1} $centimeter^{-1} \times 10^{-3}$ from 15 gram samples in 250 milliliters of distilled water for 7 hours.

taken, may have revealed if this variation was a normal occurrence. From the data it is not possible to ascertain any relation between conductance values and either total or reducing sugar content. The conductance values show no relation to hardiness as measured by winter survival. For example, Senator Dunlap, a hardy variety, has a low value and Empire, a much less dependably hardy variety, has a similar low conductance value. The late date of this sampling may have had considerable influence on these results and thus this data can only represent the conditions at this one particular time.

Table 2. The Electrical Conductance of the Leaf Exudates and the Sugar Content of the Leaves of Several Varieties of Strawberries on November 23, 1954.

Variety	Specific Conductance *	Sugar Content **	
		Reducing	Total
Sparkle	3.5	46.8	70.5
Erie	4.4	41.2	95.5
Senator Dunlap	4.5	33.2	59.7
Empire	4.7	38.4	84.3
Fairland	5.2	66.6	99.1
Vermillion	5.6	49.3	96.6
Red Rich	6.2	49.3	100.3
Premier	6.4	39.4	85.5
Red Star	6.7	41.2	51.7
Superfection	7.4	44.6	71.4
Fairpeake	8.5	55.4	121.2

* Specific Conductance in ohm^{-1} $centimeter^{-1}$ x 10^{-4} from 15 gram samples in 250 milliliters of distilled water for 7 hours.

** Sugar Content expressed as milligrams per gram of dry sample.

Further Studies of the Electrical Conductance Method

Experimental

In order to study further the effects of low temperatures and sugar accumulation in the leaves during the fall on the electrical conductance of water extracts of the leaves, four varieties were selected from field plantings for more intensive study beginning in the early fall of 1955. Long rows of excellent growth, about 100 feet long for each variety, assured a sufficient amount of leaves for representative sampling during the experimental period.

The same procedure as previously described was followed in the collection of samples from the field and in their preparation for sugar analysis. The procedure for the preparation of the leaves for conductance determinations was modified. The freezing treatment was eliminated and the ratio of fresh leaf weight to volume of water changed to 4 grams in 200 milliliters.

Since more information about the influence of sampling and handling techniques on conductance was advisable first, studies were made on several factors which might have an effect on the conductivity of the extract. The effect of using leaf fragments was studied by cutting the leaves into one-eighth inch strips immediately before immersing them in the distilled water. A second set of leaves which were not cut served as a means of comparison. The validity of the sampling technique used was tested by comparing conductance values of two leaf types with noticeable differences. Conductivity determinations were also made between replicate plantings of two varieties. Four varieties - Catskill, Senator

Dunlap, Premier, and Robinson - were selected for more intensive study. Leaf samples from these varieties were collected on September 20, October 4, October 18, November 1, and November 9. Due to the unusually cool weather in the fall of 1955 with an early approach of winter, the November 9 sample was the last which could be taken from the field. After immersing the leaves in distilled water, conductivity measurements were made on aliquots of the resulting leaf extracts at intervals of 3 1/2, 7, 14, and 24 hours.

Results and Discussion

In Table 3 the specific conductance values for the exudates of whole and cut leaves of four varieties are given. It will be noted that these values are much higher for the cut leaves. There is also more variation generally between duplicates as a result of cutting. It appears evident that lesions in the leaf surface increase the conductance considerably. Such lesions, whether visible or not, can be expected to occur in damaged leaves. In the weighing of accurate samples of whole leaves it is necessary to cut one leaf. However, this unavoidable cutting should not introduce any significant error in values.

The results of sampling leaves with different field characteristics are given in Table 4. It was observed that two types of leaves predominated in the field plots of each variety. One type was medium-sized green leaves from the initial growth or well-formed runners. The other type was composed of larger, more mature leaves mainly present in the initial growth. In all four varieties tested the conductance values were higher for the older leaves than for the green, growing leaves.

Table 3. The Effect of Cutting of the Leaf on the Specific Conductance of the Leaf Exudates of Four Varieties of Strawberries.

Variety	Leaves cut 1/8 inch strips	Whole leaves
	Specific Conductance *	
Senator Dunlap	10.5	5.9
	10.6	5.6
Premier	8.7	3.6
	7.8	3.4
Robinson	8.8	4.0
	10.1	4.3
Catskill	5.6	3.1
	10.3	3.5

* Specific Conductance in ohm^{-1} $centimeter^{-1}$ x 10^{-5} from 4 gram samples in 200 milliliters of distilled water after 7 hours.

Also there was extreme variation between duplicates in the former. This variation was considered too great to enable use of these leaves in any further study. Actually the sampling of the older leaves may more accurately represent the conditions resulting from decreased temperatures with lower photosynthetic activity and decreased metabolic rate than the green leaves.

The specific conductances of the leaf exudates from two varieties in replicate over a 24 hour period are plotted in Figure 1. Determinations are made immediately (the zero time) upon placing the leaves in the water. These values then represent the concentration of electrolytes which diffuse very readily from the leaf surface and no doubt includes traces of soil particles unavoidably present. The diffusion of electrolytic solutes from the plant tissue is shown to occur as an exponential

Table 4. The Effect of Sampling Two Types of Strawberry Leaves on the Specific Conductance of their Water Extracts.

Variety	Older Leaves	Green, growing leaves
	Specific Conductance *	
Senator Dunlap	4.4	5.9
	6.2	5.6
Premier	8.3	3.6
	7.4	3.4
Robinson	7.3	4.0
	4.7	4.3
Catskill	10.2	3.1
	7.8	3.5

* Specific Conductance in ohm^{-1} $centimeter^{-1} \times 10^{-5}$ from 4 gram samples in 200 milliliters of distilled water after 7 hours.

function of time. It exhibits a decline in rate after a period of time and would appear to reach a state of equilibrium sometime after 24 hours.

Table 5 lists the electrical conductance of the leaf exudates and the reducing and total sugar content of the leaves from the four varieties at the five sampling periods during the fall of 1955. With the exception of the Catskill variety the conductance values show a marked decrease from the first to the third sampling with a reversal of this trend in the last two samplings. On the last sampling date the conductance values are all similarly high. It will be noted that the Senator Dunlap exudates reached the lowest level of conductance among the varieties on October 18.

The reducing and the total sugar content of the leaves in all the

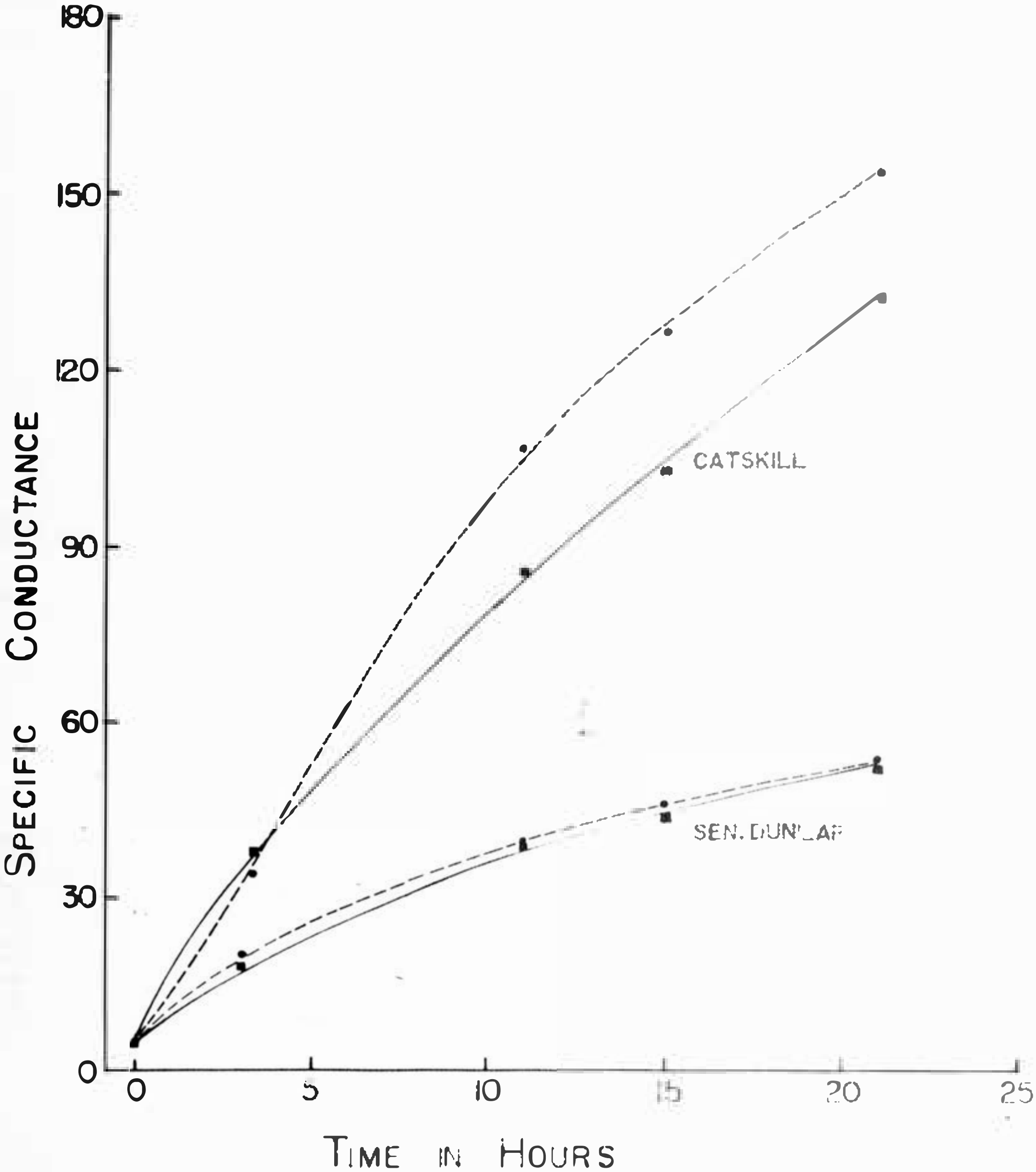

Figure 1. The Specific Conductance of the Leaf Exudates of Two Varieties of Strawberries in Replicate as Affected by Time of Exosmosis.

Specific Conductance in ohm^{-1} $centimeter^{-1} \times 10^{-6}$ from four gram samples in 200 milliliters of distilled water.

The solid lines indicate the values for the first samples and the dotted lines indicate the values for the replicates in each variety.

Table 5. The Electrical Conductance of the Leaf Exudates and the Leaf Sugar Content of Four Varieties During the Fall of 1955.

Variety	Date of Sampling	Specific Conductance *	Sugar Content** Reducing	Total
Senator Dunlap	Sept. 20	3.89	27.2	42.2
	Oct. 4	3.58	51.7	66.2
	Oct. 18	1.91	28.6	81.4
	Nov. 1	2.70	30.8	91.4
	Nov. 9	17.5	52.3	124.6
Catskill	Sept. 20	3.20	54.6	69.0
	Oct. 4	2.17	57.8	76.9
	Oct. 18	4.14	32.8	89.4
	Nov. 1	2.86	48.5	110.6
	Nov. 9	9.4	51.7	148.4
Premier	Sept. 20	3.15	59.4	78.4
	Oct. 4	2.25	74.7	97.4
	Oct. 18	2.02	32.3	65.2
	Nov. 1	2.62	48.0	105.2
	Nov. 9	18.5	43.7	112.9
Robinson	Sept. 20	4.04	55.4	79.4
	Oct. 4	2.86	75.4	101.6
	Oct. 18	2.12	36.4	67.1
	Nov. 1	3.15	48.9	116.0
	Nov. 9	13.7	53.6	117.2

* Specific Conductance in ohm^{-1} $centimeter^{-1} \times 10^{-5}$ from 4 grams in 200 milliliters of water after 7 hours.

** In milligrams per gram of dry sample.

varieties show an increase from the first to the second sampling time. The results from the third sampling date are not uniform in this respect. There is a decrease in reducing sugars in all the varieties, but an increase in total sugar in two varieties. Highest values of total sugars were reached on the last sampling date. The data from Table 5 is presented graphically in Figure 2.

Climatological data for the Brookings station from September 15 to November 9 is given in Table 6 (38). From September 15 to September 20 all the minimum temperatures were considerably above the freezing point. From September 20 to October 4 there were six days with minimum temperatures of 41° Fahrenheit (5° centigrade) or below with three of these temperatures below freezing. From October 4 to October 18 eleven days with minimum temperatures of 41° Fahrenheit or below were recorded. There were six days in this period with below freezing temperatures. During the remainder of the experimental period all daily minimum temperatures were 32° Fahrenheit or below except on one day.

It is likely that actual frost damage to the field plants resulted only when the minimum temperatures given here were considerably below freezing for a long period. Actual frost damage to strawberries does not usually occur above -5° centigrade unless these temperatures are prolonged. The first temperature below -5° centigrade occurred on October 14 and quite frequently thereafter. Prior to this time the low temperatures may have had a hardening effect on the plants. From Table 5 it is seen that the conductance values of three of the four varieties tested decreased during the period from September 20 to October 18 when, as has been noted, it is likely that the main effect of the low tempera-

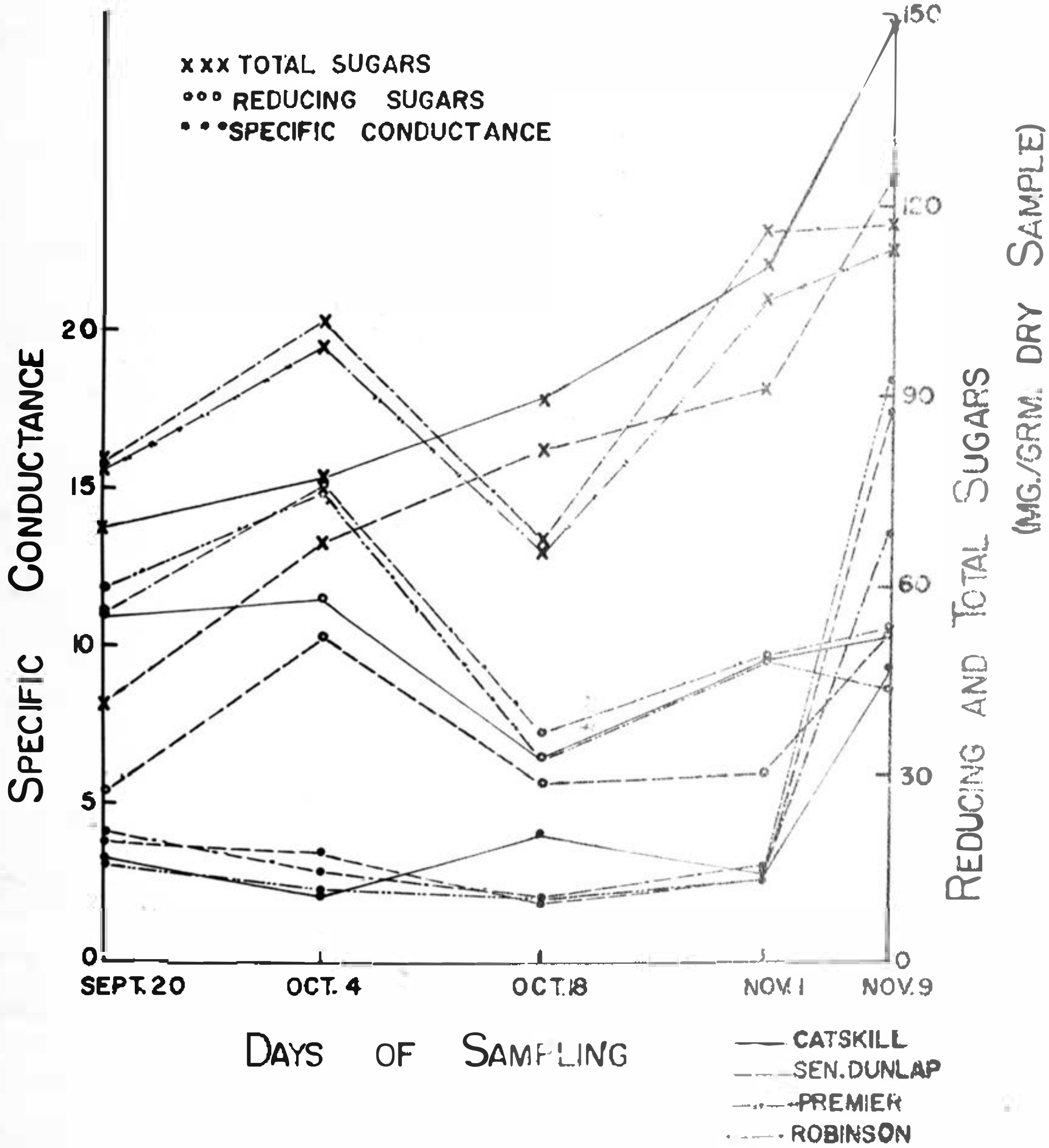

Figure 2. The Electrical Conductance of the Leaf Exudates and the Sugar Content of the Leaves of Four Varieties During the Fall of 1955.

Specific Conductance in ohm^{-1} $centimeter^{-1} \times 10^{-5}$ from four gram samples in 200 milliliters of distilled water for seven hours.

Table 6. Daily Maximum and Minimum Fahrenheit Temperatures Recorded at Brookings from September 15, 1955 to November 9, 1955.

Date	Sept.	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Maximum		97	95	95	91	80	79	71	69	59	57	50	52	69	78	75	-
Minimum		57	59	66	64	44	48	52	43	31	27	29	41	42	36	50	-
Date	Oct.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Maximum		63	70	72	70	78	73	58	73	84	83	80	73	63	57	63	
Minimum		-	40	51	54	58	40	32	29	44	40	50	34	27	21	18	
Date	Oct.	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Maximum		60	61	60	-	75	59	60	51	56	76	81	78	67	47	46	45
Minimum		29	20	18	-	27	18	41	32	7	28	32	28	35	25	21	18
Date	Nov.	1	2	3	4	5	6	7	8	9							
Maximum		42	35	39	62	64	29	29	45	51							
Minimum		21	17	4	23	29	15	15	10	30							

tures was hardening rather than injury. By November 1 the conductance values of these three varieties had all increased over the October 18 values an even greater increase was observed on the November 9 sampling. It was during the period from October 18 to November 9 that temperatures were frequently low enough to cause frost injury. Thus it appears that the conductivity of the leaf exudates is decreased when the plants are exposed to hardening temperatures, but is increased when the plants are subjected to injurious temperatures.

The electrical conductance method of Dexter and others (8,9) has been used in this study for determining frost injury in strawberry leaves.

This method measures from the water extract the concentration of the electrolytes which have diffused from the plant tissue after an interval of time. Frost damage results in an increase in the release of electrolytes from within the individual plant cells. The concentration of these electrolytes is considered proportional to the injury sustained by the plant tissue.

In the fall of 1954 the conductance measurements were made using leaves which were detached from the plant and then subjected to freezing at -10° Fahrenheit for 24 hours. The data obtained from this study then reflected the effect of this low temperature treatment and also the effect of low climatic temperatures upon the plants in the field. The study in the fall of 1955 concerned the effect of climatic temperatures alone on conductivity. However, in both instances it will be noted that initially the conductance values for each variety tested decreased. From a consideration of the minimum temperatures in the fall of 1955 only, it will be noted that this decrease occurred when temperatures were conducive to hardening. As this hardening or frost resistance is increased it is expected that the plant tissue would show an increasing ability to prevent the loss of electrolytes, which occurs in frost damaged tissue. It is thought that maximum hardening has occurred when the conductance has reached its lowest value in the plants. Senator Dunlap, the hardiest variety tested and known for its ability to harden in the fall, possessed the lowest conductivity of all varieties tested on November 10, 1954 and October 18, 1955. On the October 18, 1955 sampling, the conductance values of all varieties (except Catskill) were the lowest for that season.

After this sampling date the values increased. This increase is attributed to frost damage of the tissue with the eventual disorganization of the cell structure during dormancy. It seems that a crucial period exists when the lowest conductance values are reached; before this time hardening occurs, but after this time injury results in spite of the previous hardening effect.

From the data presented in Table 5 and also in Figure 2, no relation between the electrical conductivity of the leaf exudates and sugar content of the leaves is evident. The total sugar content of the leaves in all varieties shows an overall increase during the fall season. From the observations that conductance values have first shown a decrease followed by an increase, any simple correlation with sugar content is unlikely. If there is a correlation it may be overshadowed by other factors within the cell.

STUDIES USING GREENHOUSE PLANTS

Experimental

At the time of mulching in the fall, field plants of several varieties were transplanted into horticulture flat boxes (flats) and placed in the greenhouse having a mean temperature of 70° Fahrenheit. After an initial period of dormancy the plants began a new growth. Duplicate flats of Senator Dunlap and Robinson varieties were selected for this study. They received the same cultural practices, unless otherwise mentioned. Growth proceeded under these conditions for about two months until there were sufficient leaves available for experimentation.

In February 1956 experimental work with the Senator Dunlap plants was begun. One flat was selected for exposure to hardening temperatures; the other flat was used as a control. During the course of the study the hardened plants were inadvertently changed to another greenhouse at a lower temperature. This necessitated discontinuing this experiment, and the plants were returned to the original greenhouse.

Beginning March 27 one flat was placed in a refrigerator at 5° centigrade between the hours of 5 p.m. and 8 a.m. daily as a hardening treatment. The other flat remained in the greenhouse, but was covered during this period so that both flats received the same amount of light. From 8 a.m. to 5 p.m. both flats were kept in the greenhouse. This procedure was followed daily for one month. On April 27 both flats were placed in a freezing unit at -10° centigrade for eight hours. After this treatment they were watered, kept in the laboratory room for 12 hours and then returned to the greenhouse.

Leaf samples were first collected on April 3 after the Senator Dunlap plants had been under experiment for one week. Another collection was made on April 10. To assure a sufficient sample of leaves for the final determinations, sampling was then delayed until April 27. On that date leaves were collected before the freezing treatment and also twelve hours after the freezing treatment. All sampling was made in the afternoon prior to placing the one flat in the refrigerator except for the sampling after freezing as noted above.

For the sugar analysis one gram samples of the leaves were weighed in duplicate, washed, and cut into small pieces, and then placed in 80% hot alcohol using the procedure of the Association of Official Agricultural Chemists (2) for the preparation of the sample. The Hassid method (15,16) was used for determining reducing and total sugars. Duplicate two-gram samples of whole leaves were used for conductivity measurements except on the last sampling. In that instance it was necessary to reduce the sample one-half.

To further reduce inaccuracies, conductivity water was prepared by redistilling the regular distilled water by means of a glass-jointed condenser. This conductivity water was used for a final rinsing of the leaves. The samples were placed in wide mouth 250 milliliter Erlenmeyer flasks and 200 milliliters of the conductivity water added. The flasks were placed on a Burrell shaker at Number 2 position for twelve hours at room temperature. The flasks were removed after this time, a small portion of the water extract used for conductivity measurements, and the remainder used for the ion determinations.

For the determination of sodium and potassium ion concentrations in the exudates a Perkin-Elmer flame photometer was used. The internal standard method (30) was employed for greater accuracy. Standard concentrations of the sodium and potassium ions were prepared and their photometer readings plotted on graphs. Moisture determinations were made using a forced-air oven at 75° centigrade.

The same procedure as outlined above was used in the study of the Robinson variety of strawberries with these exceptions: The plants were transferred to an artificially lighted greenhouse with an illumination period of twelve hours. During the darkened period of twelve hours one flat was kept cool in the refrigerator at 5° centigrade; the other remained in the greenhouse.

Results

Table 7 and Figure 3 list the specific conductance and the ion concentrations of the leaf exudates of Senator Dunlap variety leaves. The data indicate that the conductance values for the samples from normal temperature plants fluctuated slightly during the course of the study with no overall increase or decrease evident. The plants exposed to hardening temperatures had more uniform and lower conductance values at each sampling. The values of both normal and hardened samples increased when the plants were subjected to freezing, but the overall effect from freezing was much less pronounced in the hardened samples.

There was considerable variation in the concentration of sodium and potassium ions in both control and hardening plants. However, the concentrations were higher in the normal plants at each sampling date. It will be noted that upon freezing there was a large increase in the

Table 7. The Electrical Conductance and the Potassium and Sodium Ion Concentrations of the Leaf Exudates from Senator Dunlap Variety Plants under Normal and Hardening Temperatures.

Date of Sampling	Normal Temperatures			Hardening Temperatures		
	Specific * Conductance	Ion Concentrations parts per million		Specific Conductance	Ion Concentrations parts per million	
		Sodium	Potassium		Sodium	Potassium
April 3	10.2	1.0	6.0	4.9	.9	1.3
	7.6	1.0	-	5.3	1.0	1.2
	7.1	1.0	5.4	5.4	.9	2.1
April 10	15.8	2.1	10.5	4.5	.3	2.2
	17.0	2.3	11.2	6.4	.6	2.3
April 27	17.7	4.1	20.3	3.3	1.2	2.7
	10.2	2.5	12.6	3.5	1.0	2.8
April 27 (after freezing)	53.9	7.3	98.	19.4	2.8	25.
	51.7	3.6	82.	14.7	2.2	9.

* Specific Conductance in ohm^{-1} $centimeter^{-1} \times 10^{-5}$ of exudates from two gram samples in 200 milliliters of conductivity water for 12 hours.

Normal temperature was 70° Fahrenheit. Hardening temperatures were 70° Fahrenheit during 9 hours of daylight and 41° Fahrenheit during 15 hours of darkness. Plants were frozen by subjecting to a temperature of 14° Fahrenheit for 8 hours on April 27.

ion concentrations, especially potassium, in both samples.

The sugar and moisture content of the leaves of Senator Dunlap variety is given in Table 8. The samples from plants at 70° Fahrenheit constantly exhibited only small changes in the amounts of reducing and total sugars during the period of the experiment. In the samples from plants at hardening temperatures, there was an increase in both reducing and total sugars during the experimental period. The moisture content

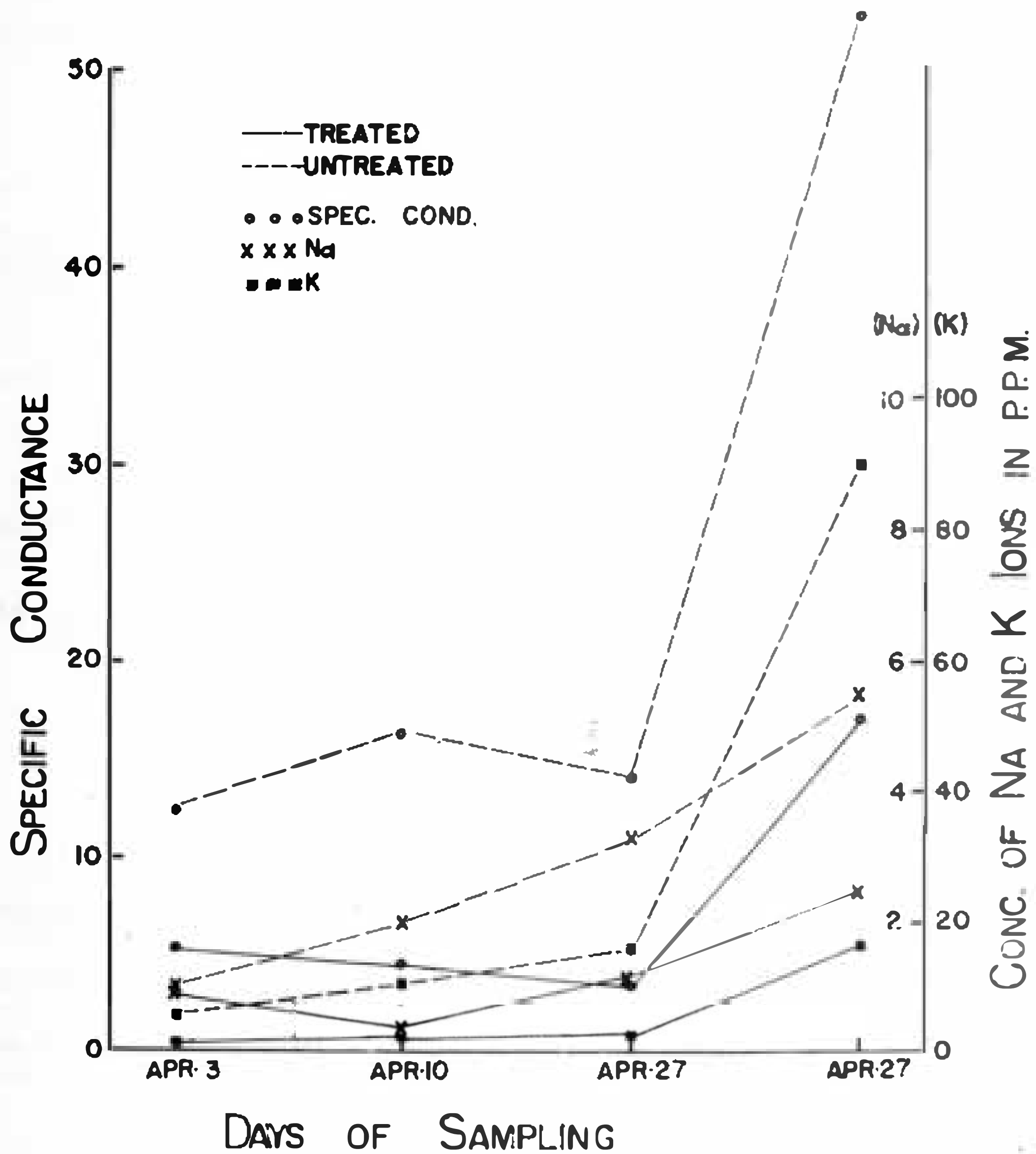

Figure 3. The Electrical Conductance and the Potassium and Sodium Ion Concentrations of the Leaf Exudates from Senator Dunlap Variety Plants under Normal and Hardening Temperatures.

Specific Conductance in ohm^{-1} $centimeter^{-1}$ x 10^{-5} of exudates from two gram samples in 200 milliliters of conductivity water for 12 hours.

Normal temperature was 70° Fahrenheit. Hardening temperatures were 70° Fahrenheit during 9 hours of daylight and 41° Fahrenheit during 15 hours of darkness. Plants were frozen by subjection to a temperature of 14° Fahrenheit for 8 hours on April 27.

Table 8. The Sugar and Moisture Content of the Leaves from Senator Dunlap Variety Plants under Normal and Hardening Temperatures.

Date of Sampling	Normal Temperatures			Hardening Temperatures		
	Sugar Content*		%Moisture	Sugar Content		%Moisture
	Reducing	Total		Reducing	Total	
April 3	13.1	17.9	67.20	14.5	18.6	66.53
	14.0	18.5	67.11	13.8	18.2	67.03
April 10	12.8	17.5	67.47	14.1	19.7	66.72
	13.5	18.1	67.12	15.2	18.8	66.42
April 27	13.9	18.1	66.78	16.5	21.2	65.10
	14.1	18.6	66.96	16.7	23.4	64.78
April 27 (after freezing)	18.7	24.7	54.22	20.1	26.0	57.21
	17.5	13.2	53.76	20.7	27.2	56.88

* Sugar content in milligrams per gram of fresh sample.

Normal temperature was 70° Fahrenheit. Hardening temperatures were 70° Fahrenheit during 9 hours of daylight and 41° Fahrenheit during 15 hours of darkness. Plants were frozen by subjecting to a temperature of 14° Fahrenheit for 8 hours on April 27.

of both normal and hardened plants showed a significant decline after freezing. This decline was greater in the normal, unhardened leaves.

In Table 9 is presented the data from the conductance and ion studies with the Robinson variety. There appears to be more uniformity between duplicates in both the normal and the hardening plants. There was a definite increase in the conductance values from May 4 to June 1 in the samples from the normal temperature plants. The parallel increase in the sodium and potassium ion concentrations during the experimental

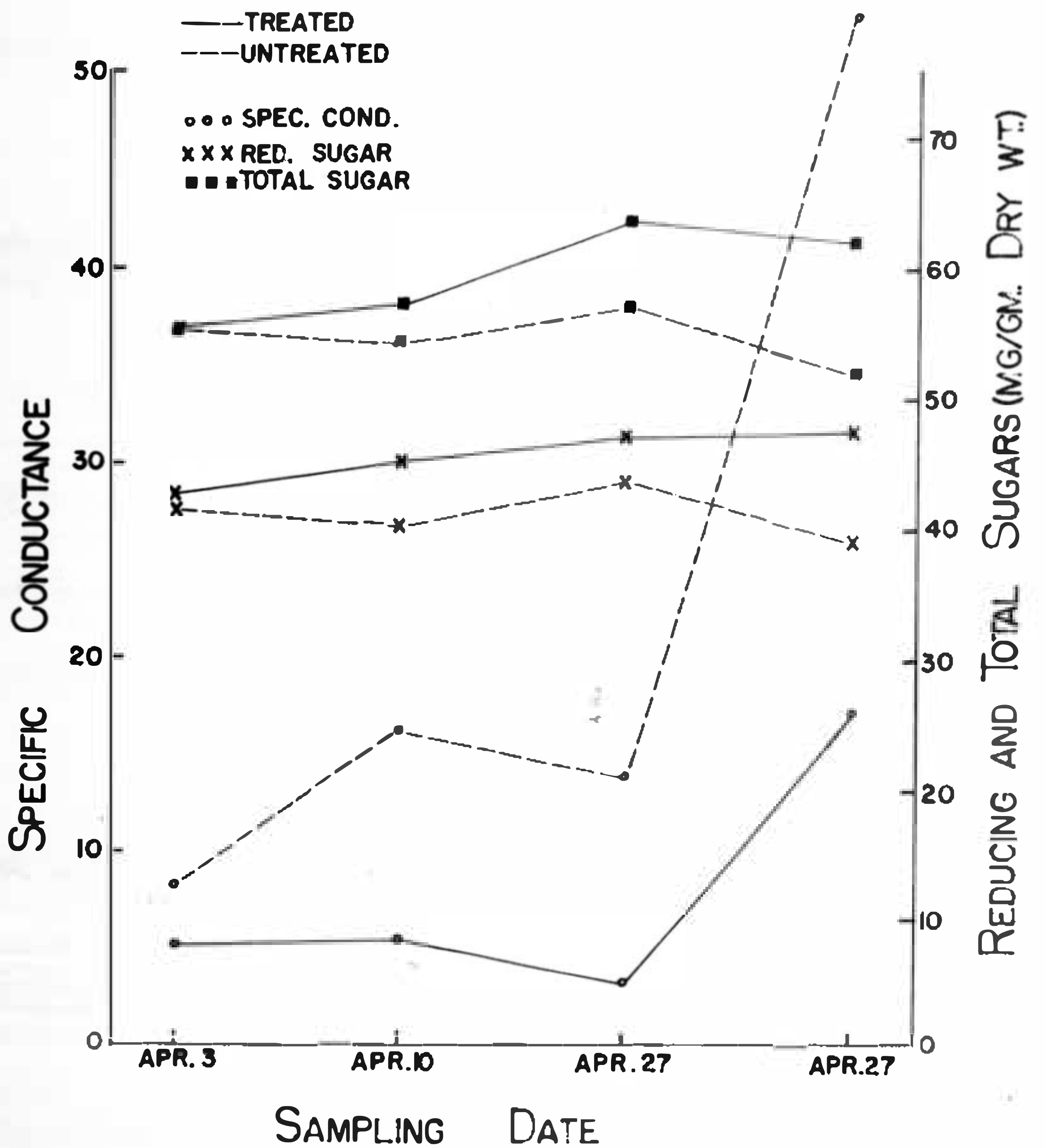

Figure 4. The Electrical Conductance of the Leaf Exudates and the Sugar Content of the Leaves of Senator Dunlap Variety Plants under Normal and Hardening Temperatures.

Specific Conductance in ohm^{-1} centimeter^{-1} x 10^{-5} of exudates from two gram samples in 200 milliliters of conductivity water for 12 hours.

Normal temperature was 70° Fahrenheit. Hardening temperatures were 70° Fahrenheit during 9 hours of daylight and 41° Fahrenheit during 15 hours of darkness. Plants were frozen by subjection to a temperature of 14° Fahrenheit for 8 hours on April 27.

Table 9. The Electrical Conductance and the Potassium and Sodium Ion Concentrations of the Leaf Exudates from Robinson Variety Plants under Normal and Hardening Temperatures.

Date of Sampling	Normal Temperatures: Specific * Conductance	Normal Temperatures: Ion Concentrations parts per million: Sodium	Normal Temperatures: Ion Concentrations parts per million: Potassium	Hardening Temperatures: Specific Conductance	Hardening Temperatures: Ion Concentrations parts per million: Sodium	Hardening Temperatures: Ion Concentrations parts per million: Potassium
May 4	4.3	.6	7.6	4.0	.8	8.8
	4.0	.6	10.1	3.5	.9	7.9
May 11	4.5	.9	8.1	3.1	.7	7.6
	5.9	.8	9.2	4.3	.7	8.1
May 18	5.1	.9	9.5	3.4	.7	7.5
	5.7	1.0	9.8	3.8	.8	7.3
May 25	6.8	1.4	10.4	4.2	.9	7.9
	6.3	1.2	11.7	4.0	.7	7.3
June 1	10.5	1.8	16.2	4.9	1.1	9.7
	7.8	1.6	14.8	4.4	1.2	10.5
June 1 (after freezing)	87.1	3.2	102.	24.8	2.9	67.
	75.3	5.0	85.	42.3	3.1	49.

* Specific Conductance in ohm^{-1} $centimeter^{-1} \times 10^{-5}$ of exudates from two gram samples in 200 milliliters of conductivity water for 12 hours.

Normal temperature was 70° Fahrenheit. Hardening temperatures were 70° Fahrenheit during 12 hours of artificial daylight and 41° Fahrenheit during 12 hours of darkness. Plants were frozen by subjecting to a temperature of 14° Fahrenheit for 8 hours on June 1.

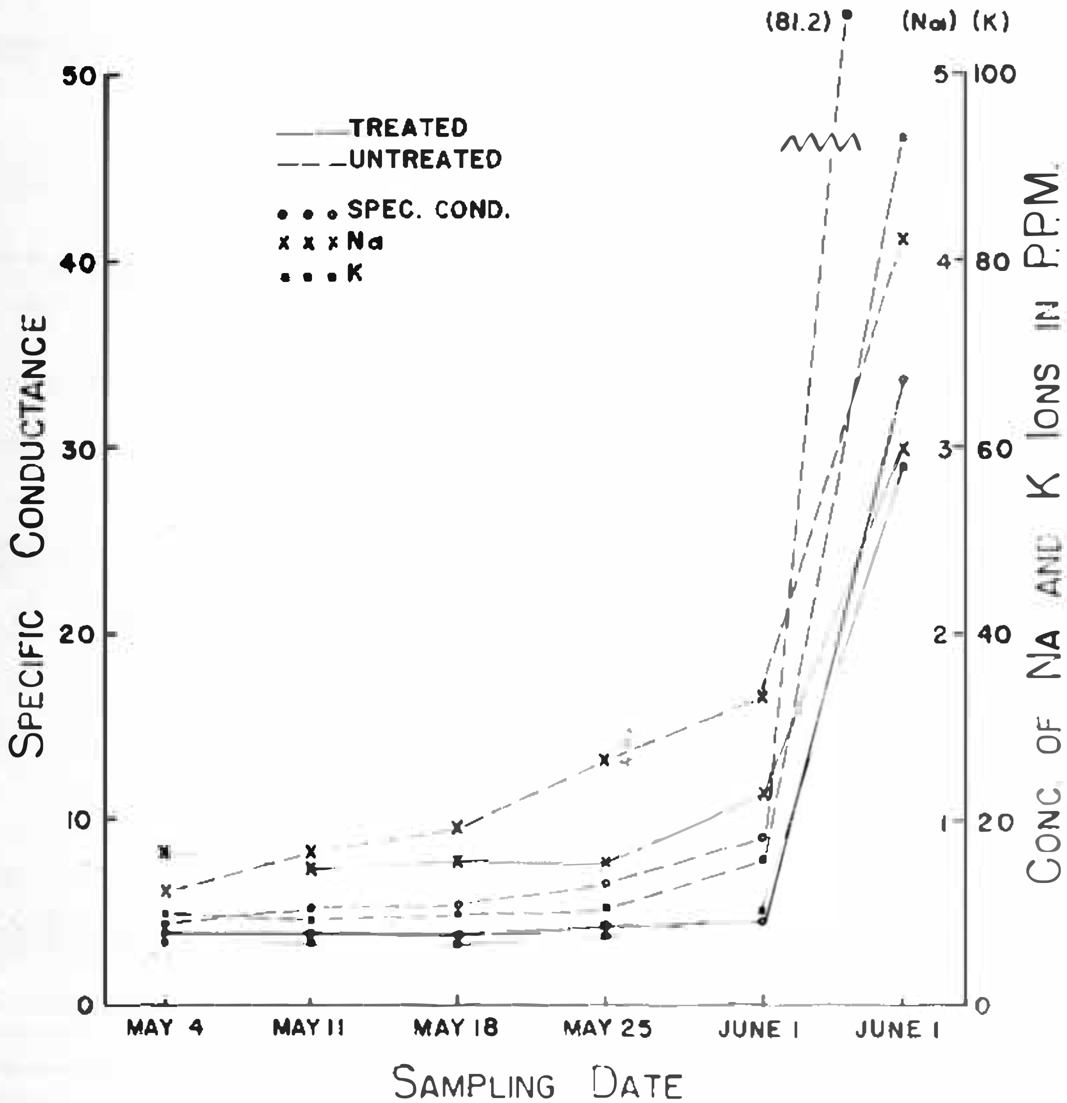

Figure 5. The Electrical Conductance and the Potassium and Sodium Ion Concentrations of the Leaf Exudates from Robinson Variety Plants under Normal and Hardening Temperatures.

Specific Conductance in ohm^{-1} centimeter-1 x 10^{-5} of exudates from two gram samples in 200 milliliters of conductivity water 12 hours.

Normal temperature was 70° Fahrenheit. Hardening temperatures were 70° Fahrenheit during 12 hours of artificial daylight and 41° Fahrenheit during 12 hours of darkness. Plants were frozen by subjection to a temperature of 14° Fahrenheit for 8 hours on June 1.

period will be observed. Figure 5 shows this increase graphically.

The sugar content of the leaves of the Robinson variety at each sampling date is presented in Table 10. The moisture content of the plants undergoing hardening showed a steady decrease from May 4 to June 1. The control or normal plants showed no definite variation of moisture content during this time. An increase in sugar content of the hardened samples is evident.

Discussion

Since studies of plants in the field lack the control of conditions necessary for many investigations, experiments were continued using plants in the greenhouse.

The identification and quantitative determination of the electrolytic substances involved in conductivity measurements of the leaf exudates would contribute to an understanding of the mechanisms involved in the hardening and frost injury processes. Considering the relatively high concentration of potassium present in plant cells, this seemed to be a logical choice for study. Although sodium is present in much smaller amounts, the small size of the sodium ion might be an important consideration in studying cell permeability.

The positive relationship between the electrical conductance and the concentration of sodium and potassium ions is evident from a consideration of the data on the Robinson variety plotted in Figure 5. While some deviation in the relation of these values to one another is present, it can logically be assumed that sodium and potassium ions are directly involved in the electrical conductance of the exudates.

Table 10. The Sugar and Moisture Content of the Leaves from Robinson Variety Plants under Normal and Hardening Temperatures.

Date of Sampling	Normal Temperatures			Hardening Temperatures		
	Sugar Content*		%Moisture	Sugar Content		%Moisture
	Reducing	Total		Reducing	Total	
May 4	12.7	14.6	67.2	12.4	15.0	67.3
	12.0	13.8	67.0	12.7	14.5	67.4
May 11	11.7	13.2	67.0	12.6	14.7	66.8
	12.1	13.3	66.5	13.0	15.1	66.6
May 18	11.1	14.1	66.2	13.6	17.6	66.5
	12.0	13.6	66.4	14.1	18.0	66.8
May 25	12.7	14.6	67.4	13.8	19.1	65.9
	11.2	14.1	67.1	13.5	18.6	66.1
June 1	12.5	15.3	65.9	14.0	19.2	65.4
	12.9	16.5	66.1	15.0	20.3	65.3
June 1	15.1	19.2	60.7	16.0	22.6	61.7
	15.8	18.5	59.9	16.5	22.0	61.0

* Sugar content in milligrams per gram of fresh sample.

Normal temperature was 70° Fahrenheit. Hardening temperatures were 70° Fahrenheit during 12 hours of artificial daylight and 41° Fahrenheit during 12 hours of darkness. Plants were frozen by subjecting to a temperature of 14° Fahrenheit for 8 hours on June 1.

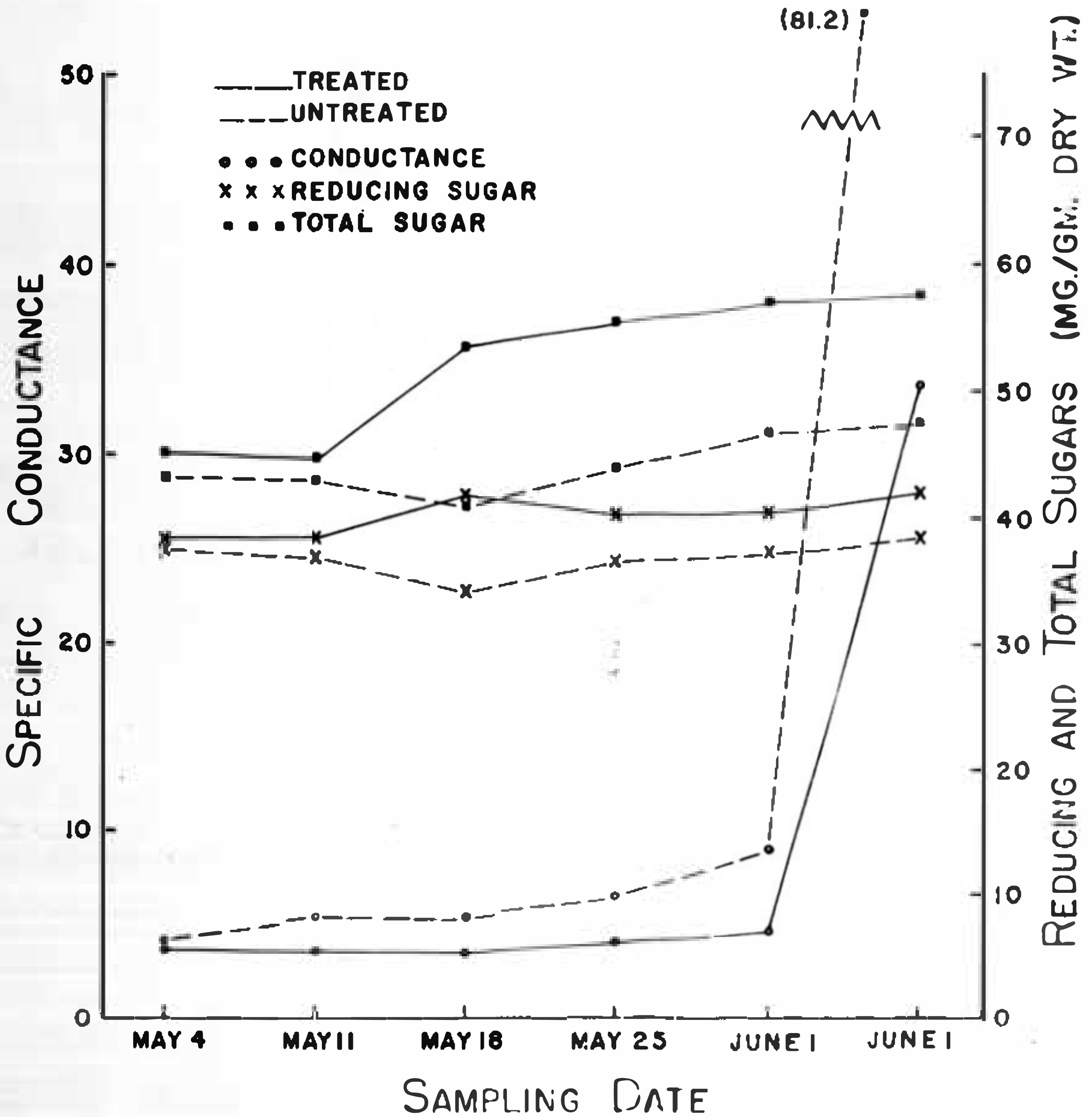

Figure 6. The Electrical Conductance of the Leaf Exudates and the Sugar Content of the Leaves of Robinson Variety Plants under Normal and Hardening Temperatures.

Specific Conductance in ohm^{-1} $centimeter^{-1} \times 10^{-5}$ of exudates from two gram samples in 200 milliliters of conductivity water for 12 hours.

Normal temperature was 70° Fahrenheit. Hardening temperatures were 70° Fahrenheit during 12 hours of artificial daylight and 41° Fahrenheit during 12 hours of darkness. Plants were frozen by subjection to a temperature of 14° Fahrenheit for 8 hours on June 1.

From the results shown in Table 7 and Figure 3 of the electrical conductance of normal and hardened plants of Senator Dunlap variety, it is noted that the specific conductance values for the samples from plants undergoing hardening decreased from April 3 to April 27, while the samples from normal plants had higher, more irregular values during this period. If it is considered that the conductance values of the normal plants represent the usual changes in the concentration of electrolytes which diffuse from the plant cell at that particular growth stage, then the effect of hardening temperatures is to decrease the concentration of these electrolytes. It is thought that this concentration is affected primarily by two factors. One of these factors is the concentration of free, mobile ions within the cell; the second is the permeability of the cell membrane to these ions. The concentration of the ions within the cell may be decreased if chemical combinations or changes in the electrical properties of the colloidal cell material renders the ions inactive. Similarly, the constituents of the cell membrane may be altered and consequently the diffusion of particles through it decreased.

From the data of the Robinson variety in Table 9 and Figure 5 it is observed that the relation between the electrical conductance of normal and hardened plant exudates is similar to that of the Senator Dunlap variety. The conductance values of the plants exposed to hardening temperatures are lower at each sample date than the normal plants. The variation between these values diverges during the period of the experiment.

The effect of freezing in both varieties as shown in Figures 3 and 5 is to increase the conductance in both normal and hardened plant samples. The increase in conductance upon freezing is only slightly greater, proportionally, in the samples of the normal plants, although the overall increase is much greater.

An increase in sugar content in the leaves of the hardened plants of both varieties occurred during the hardening process, as shown in Tables 8 and 10. The total sugar content increase was greater than the reducing sugar increase. Upon freezing, a large increase in sugar content is noted, since the values of sugar content in the tables are expressed in terms of milligrams per gram of fresh leaf. Taking into consideration the loss of moisture upon freezing, there are no significant differences as shown in figures 4 and 6 where the sugar content is expressed in terms of dry leaf weight. Variation in the moisture content of the leaves of the Senator Dunlap variety after freezing gives the false impression that the sugar content decreases upon exposure to freezing temperatures. In figures 4 and 6 the electrical conductance of the leaf exudates and the sugar content of the leaves is shown graphically for Senator Dunlap and Robinson varieties, respectively. On the basis of these results, no relation between these two quantities has been established.

The possible effect of bacterial contamination of the leaf exudates was recognized, but precautions were taken to minimize these effects by carefully washing the leaves and covering them when possible. There were no noticeable effects of contamination during the period the leaves remained in the water.

SUMMARY

A study of the electrical conductance of leaf exudates of strawberry plants in the field and under controlled conditions in the greenhouse has been made. The electrical conductance was increased by breaking the leaf surface. The electrical conductance values were observed to be a function of the time of diffusion of the leaf exudates.

During the early fall season sampling of leaves from field plants revealed that observed values of conductivity of the leaf exudates decreased among all varieties tested for a period of time. Late fall samplings showed an increase. From a study of minimum temperatures recorded during this period, it appeared that the observed decrease in conductance occurred when the temperatures were generally slightly above the freezing point, and that when the plants were subjected to injurious temperatures the electrical conductance increased.

Plants in the greenhouse exposed to hardening temperatures during the period of darkness had lower electrical conductance values than duplicate plants left at room temperature. Upon exposure to freezing temperatures the conductance values of both sets of plants increased considerably, but the increase was less in the plants which had the cold treatment. Some varietal differences were observed in the electrical conductance values.

The concentration of sodium and potassium ions in the leaf exudates showed a definite relation to the electrical conductivity values. The concentration of both reducing and total sugars in the leaves was increased by subjecting the plants to low temperatures. From this study

of both field and greenhouse plants, the changes in leaf sugar content were not related to the observed electrical conductance of the leaf exudates.

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